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ORIGINAL PAPER

Mineralization of vegetable oils used for thermal weed control in arable soils

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Abstract

Hot vegetable oil can be used for weed control as an alternative to the use of herbicides. We analysed the temporal development of vegetable oil mineralization in soil and tested the role of nutrient supply on oil mineralization. Further, we investigated the effect of oil application on mineralization of native soil organic carbon (SOC), i.e. the priming effect. In a laboratory experiment, three oil dosages (0.1, 1.0 and 3.0 ml per 35 g soil) were applied to three arable soils and soil respiration was measured hourly. Both a C3-sunflower oil and a C4-corn oil were used in order to differentiate oil-derived CO_2 from SOC-derived CO_2. The results revealed that after 42 days of incubation, 9.6 to 39.7% of the applied oil was mineralized which, however, also primed the mineralization of SOC by a factor of 2.2 to 4.2. The higher the applied oil amount, the lower was the percentage of oil-C mineralization, but the higher was the priming effect. The addition of fertilizer (0.29 mg N g^{-1} soil and 0.048 mg P g^{-1} soil) increased oil-C mineralization to 39.9 to 50.9%. We conclude that oil can temporarily accumulate in soil, especially in case of low nutrient supply. As the addition of oil stimulates SOC mineralization, a decrease of native SOC stocks may occur, which needs further quantification in long-term field experiments.

Keywords Microbial nitrogen mining · Nutrient limitation · Priming effect · Soil respiration

Introduction

Rising criticism on chemical weed control increases the need to develop ecological alternatives (Abbas et al. 2017; Ascard et al. 2007). In this regard, thermal weed control on the basis of hot vegetable oil could be an effective alternative for synthetical herbicides on arable land, especially for intra-row weed management close to crops (Peukert 2018). Small amounts of 0.1 to 1.0 ml of vegetable oil per weed are heated and applied selectively on single weeds (Peukert 2018). In this case, oil acts as a carrier of thermal energy and destroys plant cells by protein denaturation (Peukert et al. 2017; Zhang et al. 2012). Thereby, oil reaches the soil directly by dropping or flowing down or indirectly by decomposition of the killed weed. After developing this practice in laboratory and greenhouse successfully, the next step will be experiments in field (Peukert 2018). Generally, vegetable oil is not classified for physical or health hazards according to the Globally Harmonized System of Classification and Labelling of Chemicals (e.g. http://www.btps.ca/documents/general/Vegetable_Oil.pdf 2011) and is already used as a carrier of other pesticides (Malkomes 2004). Yet, little is known about the mineralization rate of pure oil in soils and its determining factors. As an accumulation of oil in soils may have negative consequences for plant germination (Tamada et al. 2012) and physical soil properties like water repellence, infiltration, and aeration (Klamerus-Iwan et al. 2015; Sonnleitner et al. 2003),

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knowledge about the mineralization rate is necessary for recommendations of acceptable application rates.

It is generally assumed that vegetable oil, like rapeseed oil, is easily degradable and enzymes for its mineralization are ubiquitously existing (Cornish et al. 1993; Malkomes 2005; Zhang et al. 1998). Under aerobic conditions, vegetable oil in soils is completely mineralized to CO_2, H_2O and inorganic ingredients (Cornish et al. 1993). Several authors assessed the mineralization rate of vegetable oil in soil and the consequences of oily substrates on soil microorganisms (Malkomes 2004, 2005; Sonnleitner et al. 2003). Yet, the applied dosages were often different to those applied during thermal weed control (e.g. 5 ml and 10 ml per 100 g of soil were used in Sonnleitner et al. (2003), which is considerably higher than the recommended application of 0.1 ml per weed), oils were mixed with other substances (Malkomes 2004, 2005), or olive oil mill wastewater was considered (Peikert et al. 2017). Thus, it is necessary to test the mineralization rate of purely and punctually applied oil as it is used for thermal weed control.

Vegetable oil is rich in C but comparatively poor in nutrients like N and P (Sonnleitner et al. 2003). Hence, an accumulation of vegetable oil in soils may induce a long-term microbial N incorporation (Malkomes 2004, 2005). Previous studies indicated that the mineralization rate of C-rich substrates depends on the availability of nutrients in soil (Meyer et al. 2018; Nordgren 1992). In this regard, it appears reasonable to suggest that nutrient supply also regulates the mineralization rate of oil in soils and that a deficiency of nutrients may retard or even limit the mineralization of oil.

On the other hand, it has been shown that the input of labile C (i.e. here vegetable oil) not only increases the demand of microorganisms for N but also provides energy for the breakdown of soil organic matter (SOM), which contains the required N ('microbial N mining theory', Craine et al. 2007). Such a stimulation of native SOC mineralization caused by labile C supply is commonly referred to as a positive priming effect (Kuzyakov et al. 2000). As several studies reported that positive priming is more pronounced under conditions of N deficiency (Blagodatskaya et al. 2007; Fontaine et al. 2011), the priming effect has increasingly been interpreted as a response of microbes to unsatisfied nutrient demand (Murphy et al. 2015). Hence, it seems reasonable to assume that the addition of oil stimulates the turnover of native SOC depending on the soil nutrient status. As previous studies focussed on the effect of oil addition on total CO_2 release without distinguishing oil-derived from SOC-derived CO_2 (e.g. Malkomes 2006; Sonnleitner et al. 2003), an effect of oil addition on native SOC mineralization has, to our knowledge, not been investigated yet.

This study aims at investigating the temporal dynamics of vegetable oil mineralization in soils and the response of native SOC turnover to oil additions. As oil provides only low nutrient amounts for soil microorganisms, we assume that the mineralization rates of oil depend on nutrient supply and that fertilization increases the oil mineralization rate. Further, we assume that oil addition necessitates microbial nutrient mining, thereby increasing the priming effect, i.e. the mineralization of native SOC. To unravel these hypotheses, we added different dosages of naturally ^{13}C-labeled vegetable oil with and without additional nutrient supply to three different soil samples, incubated them for 42 days at 22 °C, and continuously measured CO_2 release.

Material and methods

Soil sampling

Three soil samples with different SOC and nutrient contents were taken from arable fields in North Rhine-Westphalia, Germany (Table 1). Only C3 plants had been cultivated on these fields for at least the last decades, resulting in low $\delta^{13}C$ values (Table 1). The altitude of the study sites ranged between 33 and 190 m above sea level. Samples were taken in spring. The fields were cultivated with winter wheat, sugar beet and one was prepared for sowing sugar beet. Basic soil properties are summarized in Table 1. At each sampling site, one sample was taken from 0 to 30 cm depth (Ap horizon) using a spade. The field-moist soil was stored at 7 °C for 1 week and sieved to 2 mm, i.e. all results refer to the fine soil < 2 mm. Sieved soils were divided into three parts: one was dried at 40 °C for chemical analysis, one was stored frozen (− 18 °C) until the start of the incubation experiment, and one was stored field moist at 7 °C for determination of water holding capacity.

Table 1 Properties of the soil samples (arable soils, Ap horizon)

	Soil 1	Soil 2	Soil 3
Sand (%)	15.0	72.3	11.9
Silt (%)	63.4	20.3	62.7
Clay (%)	20.7	6.0	22.6
N-total (g kg^{-1} soil)	2.05	1.45	1.53
CAL-P (mg kg^{-1} soil)[a]	140	266	64.3
CAL-K (mg kg^{-1} soil)[a]	314	313	318
SOC (g kg^{-1} soil)	21.6	16.8	13.8
C/N ratio	10.5	11.6	9.0
pH in $CaCl_2$	6.2	5.8	6.6
$\delta^{13}C$ (‰)	− 27.1	− 26.8	− 27.2

[a] Plant available concentrations, extraction with citrate -acetate -lactate mixture

Soil physical and chemical analysis

Texture was determined by a combination of wet sieving (sand fraction) and sedimentation (silt and clay fraction) after Köhn (ISO 11277 2002). The total C and N contents of milled soils were determined by elemental analysis (Vario MICRO Cube, Elementar, Hanau, Germany; ISO 10694 1995). All samples were free of carbonates, so total C corresponded to SOC. Plant available P and K were extracted by using the calcium–acetate–lactate method (Schüller 1969; Zbíral 2000). The concentration of P was measured photometrically with the molybdenum blue method (Murphy and Riley 1962) and that of K by atomic absorption spectroscopy (AAS). The pH value was measured in a 0.01 M $CaCl_2$ solution (soil:solution ratio of 1:2.5). Water holding capacity was determined on field moist soil by placing soil into funnels and submerging in water for 30 min. Afterwards, soil was allowed to drain for 24 h, weighed and dried at 105 °C.

Incubation experiment

The sieved and frozen soil samples were defrosted for 2 days at 7 °C. Each soil sample was rewetted or dried to 45% of its water holding capacity, which warrants a sufficient and standardized water availability (ISO/DIS 17155 2001). Each sample was homogenized using a mixer and divided into 30 to 36 plastic vessels, each corresponding to 35 g of dry soil. The samples were then pre-incubated at 22 °C for 96 h to level out effects of mixing and water addition and to stabilize the respiration rate (Blagodatsky et al. 2000).

After pre-incubation, oil was added to the soil samples. To differentiate between CO_2 that evolved from the applied oil and CO_2 that derived from native SOC, we used commercially produced oil from corn ('C4 oil') that differs from the native SOC in terms of its $\delta^{13}C$ value (Bol et al. 2003; Kuzyakov and Bol 2006 for distinguishing slurry-derived CO_2 from SOC-derived CO_2 or Nottingham et al. 2009; Meyer et al. 2017 for distinguishing sugar-derived CO_2 from SOC-derived CO_2). The $\delta^{13}C$ value of the C4 oil was − 16.65‰. The differentiation of oil- and SOC-derived CO_2 based on the $\delta^{13}C$ value of released CO_2 would be straightforward if no isotopic fractionation occurred during mineralization. However, several studies indicated that isotopic fractionation does occur (e.g. Fernandez et al. 2003), which may lead to immense errors in the quantification of CO_2 sources. To account for isotopic fractionation of the C4 oil, we followed suggestions of Bol et al. (2003) and conducted the same experiment also with a C3 control. For this, oil with a similar $\delta^{13}C$ value as native SOC is required as C3 control. Therefore, we used a C3 sunflower oil, which was mixed with the C4 corn oil at a ratio of 5:1 to obtain the required isotope ratio (− 27.19‰, 'C3 oil'). Pre-tests revealed no difference in soil respiration rates between the two oils. The $\delta^{13}C$ value of oil samples was determined by isotope ratio mass spectroscopy (IRMS, Thermo Delta V Advantage, Thermo Electron, Bremen, Germany) after weighing 0.5 mg of oil and 0.1 to 0.2 mg of C-free 'Chromosorb' into tin cups; the latter was used to absorb the oil. Total N contents were determined by elemental analysis as already reported. Total P was determined by ICP-OES (Horiba Jobin Yvon, Ultima 2) after digestion with concentrated nitric acid. Both oils had a C content of 79.6%, a N content of < 0.007% and a P content of 2.9 mg P kg^{-1} oil (C3 oil) and 3.25 mg P kg^{-1} (C4 oil), respectively.

The oils were applied with a nozzle according to Peukert et al. (2017). The nozzle height above soil was 15 cm. During application, the oil was mixed constantly with the soil using a vortex shaker. Three different dosages were applied (see Table 2): five drops per vessel, i.e. per 35 g soil ('low dosage', 0.1 ml oil $vessel^{-1}$; ≈ 3×10^{-3} ml g^{-1} soil), 50 drops per vessel ('medium dosage', 1.0 ml oil $vessel^{-1}$; ≈ 0.03 ml g^{-1} soil) and 150 drops per vessel ('high dosage', 3.0 ml oil $vessel^{-1}$; ≈ 0.09 ml g^{-1} soil). The dosages 'low' and 'medium' were chosen in order to simulate realistic dosages: Peukert (2018) proposed the punctual application of 0.1 ml to maximal 1.0 ml oil to a single weed plant. Thus, assuming that one weed plant grows on the vessel area of 28.3 cm^2 the dosages low and medium represent the lower and upper range of recommended dosages. The high dosage of 3.0 ml oil was conducted to simulate effects like inadvertent release of oil or a very high weed cover. The applied oil had a temperature of 22 °C. Note that we also tested the effect of higher oil temperatures during application (100 °C). As results were not significantly different between 22 and 100 °C (Table S1), we concentrate here on reporting only results of the 22 °C treatments.

To test effects of nutrient limitation on oil mineralization, the oil dosages low and medium were conducted in combination with an additional nutrient supply. The variant with 'high' oil amount was excluded because the stimulation of microorganisms was expected to be too high to allow for a continuous measurement of CO_2 release. The applied powdery nutrient mixture contained 47.88 mg $(NH_4)_2SO_4$ (0.29 mg N g^{-1} soil), 7.42 mg KH_2PO_4 (0.048 mg P g^{-1} soil), and 154.7 mg talcum powder per vessel. These amounts of nutrients were proposed by ISO/DIS 17155 (2001), and talcum powder was used as an inert carrier (Anderson and Domsch 1978). The powder was mixed to the soil immediately before addition of oil using a vortex shaker.

As it is known that microbial nutrient demand forces the acquisition of nutrients from SOM, we tested whether this 'nutrient mining' is as effective in providing nutrients required for oil mineralization as the addition of mineral nutrients. To test this, we repeated the abovementioned nutrient addition to selected vessels after finishing the incubation experiment at day 42, i.e. to vessels that already received nutrients at the beginning of the experiment (second nutrient addition) and to vessels that did not receive nutrients at the beginning (first

Table 2 Treatments of the incubation experiment including maximum hourly respiration rate (CO_{2max}) and time until the first respiration peak is reached (t_{CO2max}) ($n = 2$ or 3)

Treatment	Amount of added oil per vessel	Nutrient addition	CO_{2max} (mg CO_2 h^{-1}) Mean ± SD	t_{CO2max} (h) Mean ± SD
1	Low (0.1 ml)	No	0.4 ± 0.2	77 ± 45
2	Medium (1.0 ml)	No	1.5 ± 0.6	37 ± 5
3	High (3.0 ml)	No	1.5 ± 0.6	35 ± 5
4	Low (0.1 ml)	Yes	0.7 ± 0.1	64 ± 11
5	Medium (1.0 ml)	Yes	5.9 ± 1.4	60 ± 1
6	Control (none)	No		
7	Control (none)	Yes		

nutrient addition). If nutrient mining by microbes releases the demanded amount of nutrients, CO_2 release would not increase upon the late nutrient addition to vessels that had not received nutrients at the beginning.

We also conducted a control treatment, which received neither oil nor nutrients, and a treatment, which received nutrients but no oil. Thus, every soil sample was split into seven treatments with two (for two soils and the dosage low) or three analytic replications each (Table 2).

Soil microbial respiration was measured using an automated respirometer that allows incubating 95 samples in parallel (Respicond VIII, Nordgren Innovations AB, Sweden). The plastic incubation vessels are arranged in a water bath, which ensures a constant soil temperature of 22 °C. The system provides a continuous measurement of CO_2 evolution by trapping CO_2 in potassium hydroxide (KOH) (Nordgren 1988). Soil respiration was measured hourly for 1008 h, i.e. for 42 days. As the KOH solution has a finite capacity to capture CO_2, it was replaced several times during the incubation period in case that about half to three-quarter of the capacity was reached. During replacement, aeration of the samples and equilibration with ambient O_2 was allowed.

From the respiration curve, the maximum hourly respiration rate (respiratory peak, CO_{2max}) was derived as an indicator of nutrient availability (Meyer et al. 2017; Nordgren 1992). In case that two respiratory peaks developed during incubation (cf. Fig. 1), CO_{2max} describes the height of the first peak, which usually occurred within the first 130 h after oil addition. Further, we calculated the time needed until this peak was reached (t_{CO2max}), which indicates the viability of microorganisms (ISO/DIS 17155 2001). Total cumulative amounts of released CO_2 during the entire incubation period (CO_{2cum}) were calculated by summing up all hourly values of CO_2 release.

Quantification of oil- and SOC-derived CO_2

To obtain the $\delta^{13}C$ value of released CO_2, which allows distinguishing SOC-derived from oil-derived CO_2, the CO_2 captured within KOH had to be precipitated. The KOH solution was replaced several times during the incubation period (see above), and the replaced solution of every vessel was collected in airtight bottles. After completion of the incubation experiment, the bottles (each of them containing the entire KOH collected from each vessel) were shaken and excess $BaCl_2$ solution (3 ml of 1 M $BaCl_2$) was added to a 20 ml aliquot of the replaced KOH. The addition of $BaCl_2$ induced an immediate precipitation of $BaCO_3$. The solution was vacuum-filtered with glass fibre filters and rinsed with distilled water. The remaining $BaCO_3$ was dried at 40 °C and homogenized by grinding. About 1.6 mg of the precipitated $BaCO_3$ was weighed into tin cups, corresponding to 100 μg C per tin cup. Measurements of $\delta^{13}C$ were conducted with isotope-ratio mass spectrometry (IRMS, Delta V Advantage Thermo Electron, Bremen, Germany).

The difference in the $\delta^{13}C$ value between CO_2 respired after additions of C3 oil and C4 oil in each sample, and treatment was used to quantify the proportion of oil- and SOC-derived CO_2 to total CO_2 release (Bol et al. 2003; Eq. 1). $\delta CO_{2\backslash C4}$ and $\delta CO_{2\backslash C3}$ are the $\delta^{13}C$ values of the CO_2 of the respective variant, whereas the subtraction of the C3 variant corrects the microbial isotope fractionation and adjusts this effect. The variables δC_4 and δC_3 are the $\delta^{13}C$ values of the applied C4 oil (δC_4) and C3 oil (δC_3).

$$\text{Proportion of oil derived } CO_2 \text{ to total } CO_2\ (\%) = \frac{\delta CO_{2\backslash C4} - \delta CO_{2\backslash C3}}{\delta C_4 - \delta C_3} \times 100 \quad (1)$$

Absolute amounts of CO_2 derived from oil during the whole incubation time were calculated by Eq. 2, i.e. the proportion of oil-derived CO_2 from Eq. 1 was multiplied with the respective cumulative amount of CO_2 (CO_{2cum}).

$$\text{Absolute amount of oil derived } CO_2\ (\text{mg } CO_2\ \text{vessel}-1) = \frac{\delta CO_{2\backslash C4} - \delta CO_{2\backslash C3}}{\delta C_4 - \delta C_3} \times CO_{2cum} \quad (2)$$

The amount of C applied by the oils depended on the variant. The estimated oil density at 22 °C of 0.91 g ml^{-1} (following Esteban et al. 2012) was multiplied by the oil amount (ml) of each treatment (oil amount) and the C-proportion of 79.6% of the oils in Eq. 3.

$$\text{Amount of added C via oil addition}\left(\text{mg C vessel}^{-1}\right) = \text{oil amount} \times 0.91\frac{\text{g}}{\text{ml}} \times 0.796 \times 1000 \quad (3)$$

Subsequently, the mineralization of oil was expressed as the loss of oil-C after 42 days of incubation, which was calculated according to Eq. 4, based on a proportion of 27.3% C of CO_2. Hence, the CO_2 amount derived from oil (Eq. 2) was multiplied by 0.273 and divided by the respective amount of added C (Eq. 3). Thus, we got the relative oil mineralization within the incubation period.

$$\text{Mineralization of oil–C after 42 days (\%)} = \frac{\text{absolute amount of oil derived } CO_2 \times 0.273}{\text{amount of added C}} \times 100 \quad (4)$$

The amount of SOC-derived CO_2 was calculated by subtracting the amount of oil-derived CO_2 (Eq. 2) from the total amount of CO_2 (CO_{2cum}). The priming effect, i.e. the extra amount of CO_2 that was derived from SOC compared to the control was calculated according to Eq. 5, i.e. by dividing the amount of CO_2 respired from native SOC after oil addition by the amount of CO_2 respired in the control (CO_{2cum} Control), which received neither oil nor nutrients or no oil but nutrients (Bol et al. 2003).

$$\text{Priming effect} = \frac{\text{SOC derived } CO_2}{CO_{2cum}\ \text{Control}} \quad (5)$$

Statistical analyses

The average of the two to three replicates from each sample and treatment was used for statistical analyses. After each replacement of KOH, the values of the first 2 h afterwards were deleted because O_2 and CO_2 from atmosphere came into the vessels. To investigate differences between the treatments, we conducted a three-way ANOVA with the factors oil dosage, fertilization, and sampling site, whereas the latter was considered as block effect. We also tested the interaction between oil dosage and fertilization. In case of significant effects ($p < 0.05$), Tukey HSD post-hoc test was carried out to compare differences between treatments. We checked for normal distribution of the data with Shapiro–Wilk test and for variance homogeneity with Levene's test. However, due to the small number of independent soil samples ($n = 3$), statistical test results should not be overinterpreted. Yet, they are useful to indicate tendencies. All statistical analyses were performed with R (version 3.2.3, R Core Team 2013).

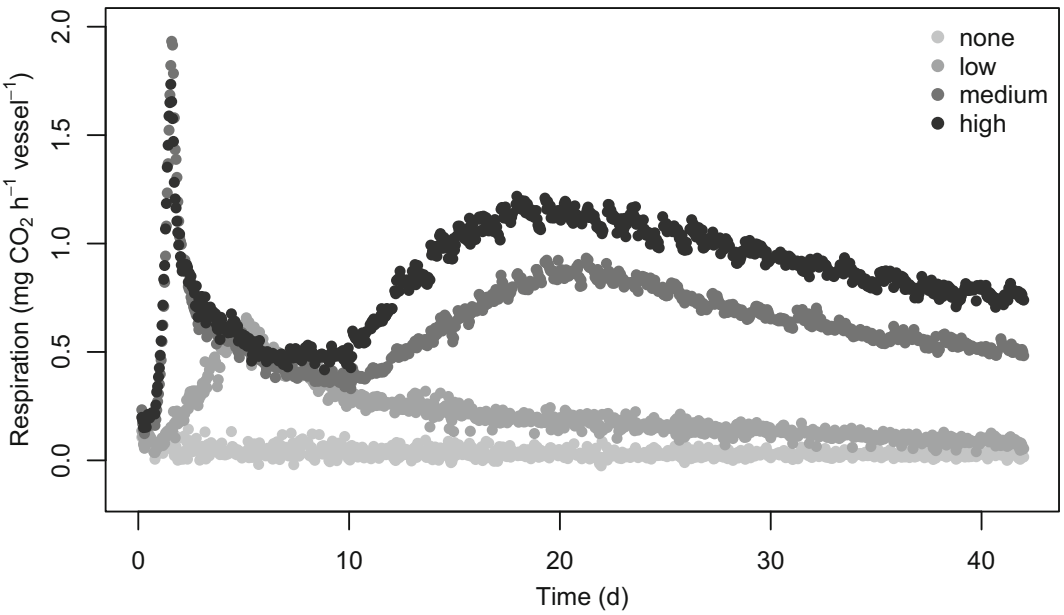

Fig. 1 Hourly CO_2 release during the 42 days incubation period as affected by oil dosage (without nutrient additions). Results are shown exemplarily for soil 2

Results

Total CO_2 release after oil addition

The addition of oil induced an exponential increase of soil respiration in all samples (see Fig. 1). The maximum respiratory peak of this exponential increase (CO_{2max}) within the first 130 h after oil addition was significantly larger in the medium-dosage treatment (1.5 ± 0.6 mg CO_2 h^{-1}) than in the low-dosage treatment (0.4 ± 0.2 mg CO_2 h^{-1}; see Table S2 for detailed ANOVA output). In the high-dosage treatment, however, the maximum respiratory peak did not further increase (Table 2). Likewise, the time needed to reach the first peak (t_{CO2max}) decreased when the amount of oil exceeded the minimum amount, without significant differences between medium-dosage and high-dosage treatments (Table 2). After reaching CO_{2max}, the hourly respiration rate continuously decreased in the low-dosage treatment but developed a second respiratory peak in the medium-dosage and high-dosage treatments (Fig. 1).

Total CO_2 release was significantly higher after oil addition compared to the control (Fig. 2a); treatments with low dosage released more than five times more CO_2 than samples without oil. The higher the oil dosage, the more CO_2 was released during the whole incubation time of 42 days (Fig. 1). Yet, the cumulative amount of CO_2 release did not increase proportionally with increasing oil dosage (Fig. 2a).

Fertilization had no significant influence on total CO_2 release (CO_{2cum}) in the treatments without oil application (Fig. 2a). Adding nutrients together with the oil induced slight increases of the peak respiration rate (CO_{2max}) and total CO_2 release (CO_{2cum}) in the low-dosage treatment, which were, however, not significant. In contrast, the effect of nutrient additions on CO_{2cum} and CO_{2max} was significant in the medium-dosage treatment: CO_{2cum} was 1.9 times magnified compared to the variant without nutrients and CO_{2max} increased by a factor of 3.9 (see Fig. 2a and 3). A second nutrient addition at day 42 did not result in a considerable stimulation of CO_2 release (Fig. 3). In contrast, samples that received nutrients for the first time at day 42 developed a pronounced respiratory peak (Fig. 3).

Mineralization of oil

As already observed for CO_{2cum}, oil-C mineralization did not increase proportionally with oil dosage: the triplication of oil dosage from the medium-dosage to the high-dosage treatments did not result in a 3-fold increase of oil-C mineralization but only increased by a factor of 1.3 (Fig. 2b). As a result, percentage mineralization of oil-C after 42 days of incubation decreased with increasing oil dosage. This decrease was evident in all samples, although the difference was only significant between the low-dosage and the medium-dosage treatments and the low-dosage and high-dosage treatments (Fig. 2b).

Fertilization increased absolute amounts and the percentage oil-C mineralization (Fig. 2b) in both tested dosages, but the effect was only significant for the medium-dosage treatment: here, the mineralization of oil-C increased 1.9 times by fertilization (Fig. 2b). By pairwise comparing the mineralization of each soil sample with and without nutrients, oil-C mineralization was always higher after nutrient addition.

Effect of oil addition on soil organic C mineralization

The addition of oil increased the mineralization of SOC in all treatments compared to the control, i.e. we observed that positive priming effects > 1 occurred (Fig. 2c). The priming effect increased with increasing amounts of oil addition, though this difference was statistically not significant (Fig. 2c).

The addition of nutrients resulted in a significantly higher priming effect in the medium-dosage treatment but had no significant effect on priming in the low-dosage treatment (Fig. 2c).

Discussion

Mineralization of oil

All tested soils showed an increased CO_2 release after oil addition. The addition of C via oil resulted in an exponential increase of soil respiration. This immediate and exponential respiration response to oil addition is similar to what is usually observed after addition of highly labile substrates (e.g. glucose in Blagodatsky et al. 2000; Nordgren 1992). Hence, we assume that vegetable oils contain besides recalcitrant compounds also considerable amounts of easily mineralizable C.

Various publications state that vegetable oils are a mixture of different substances, which are completely mineralizable by microorganisms (e.g. Zhang et al. 1998). Yet, in our experiments after 42 days of incubation at 22 °C and optimal conditions of soil moisture (ISO/DIS 17155 2001), oil-C was mineralized by 64% at maximum (soil 2, addition of 0.1 ml oil, with nutrient addition; Fig. 2b). The amount of mineralized oil-C did not increase proportionally with increasing amounts of oil addition. Hence, the proportion of mineralized oil-C decreased with increasing amounts of added oil. This indicates that the overall mineralization of oil is limited at high dosages of application, which may lead to a temporary accumulation of oil in soils.

The limitation of oil mineralization at high dosages might be caused by a lack of available nutrients. There are two indications that nutrient supply regulates the mineralization rate of

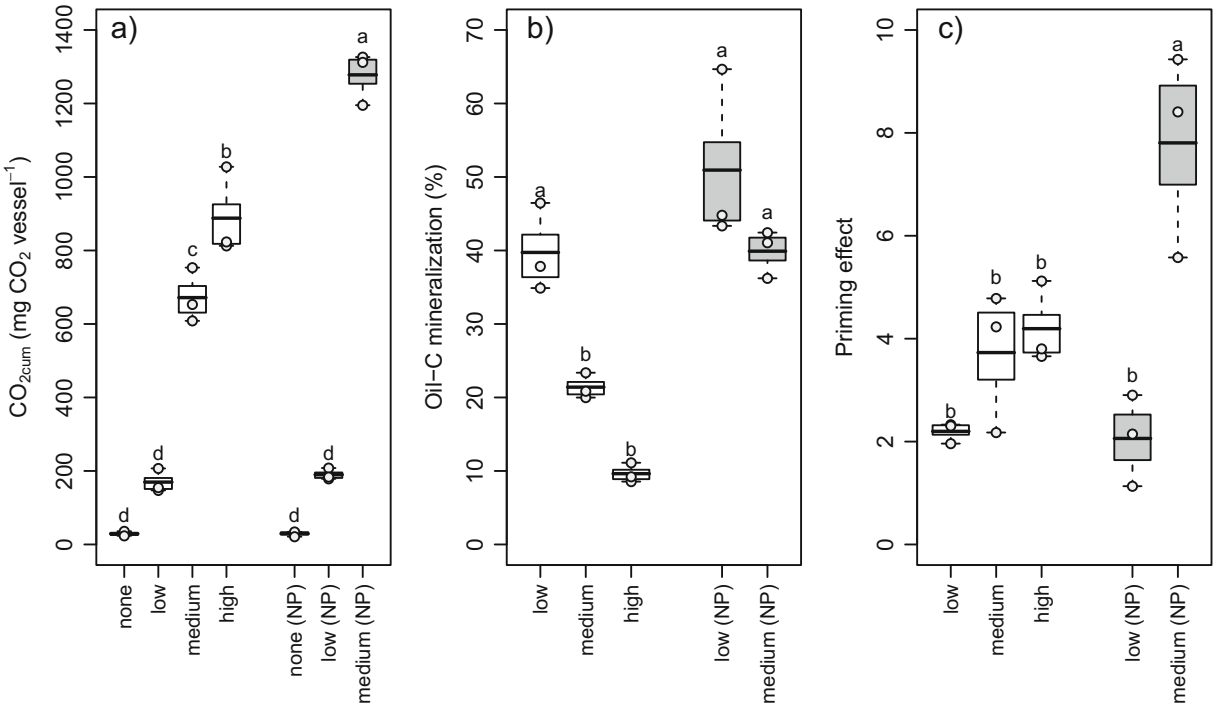

Fig. 2 Results of the incubation experiment. **a** Cumulative CO_2 release, CO_{2cum}. **b** Oil-C mineralization. **c** Priming effect depending on the applied oil dosage and nutrient supply. Treatments with nutrient addition are highlighted in grey. The light grey circles show the results of each individual sample

vegetable oil in soils. (1) The height of the respiratory peak (CO_{2max}) increased from the low-dosage to the medium-dosage treatments but did not increase any further in the high-dosage treatment. The height of a respiratory peak after the addition of labile C has often been interpreted as an indicator for the amount of immediately available nutrients because microbial growth continues until either the labile parts of the added substrates or the required nutrients are consumed (e.g. Nordgren 1992). Hence, it appears reasonable to speculate that nutrient supply was sufficient at low dosages but did not meet microbial demand at higher application dosages. (2) The addition of nutrients together with oil application increased CO_{2cum} and the proportion of mineralized oil-C especially in the medium-dosage treatment (Fig. 2a, b). A stimulation of oil mineralization by nutrient addition was also observed by Malkomes (2004). Hence, sufficient nutrient supply increases oil mineralization rates.

If not enough nutrients are available, microorganisms may also be able to acquire the required nutrients from SOM, which is referred to as 'microbial nutrient mining' (Craine et al. 2007; Moorhead and Sinsabaugh 2006). The development of a second respiratory peak may point to this mechanism (Beck 1991; Meyer et al. 2017; Teklay et al. 2006), although other mechanisms like changes in the microbial community composition could also explain this phenomenon. To test whether such nutrient mining may release sufficient amounts of nutrients for oil mineralization, we applied nutrients after finishing the experiment at day 42 to some vessels that did not receive nutrients at the beginning. The results revealed that nutrient additions induced an increased CO_2 release and a respiratory growth phase even after day 42 (Fig. 3), grey symbols. This indicates that oil mineralization was still nutrient limited in these samples and that nutrient mining, if it occurred, did not release enough nutrients to meet microbial demand. Hence, at higher oil application dosages, the required nutrient demand only seemed to be met in samples that received additional nutrients (Fig. 3, black symbols).

We are aware that oil can create impermeable layers in soil and may act as a diffusion barrier for oxygen (Klamerus-Iwan et al. 2015), thereby potentially also creating anaerobic conditions. We therefore cannot exclude that organic intermediates are produced in addition to CO_2, which, however, could not be measured using the Respicond. This could lead to underestimation of oil and SOC mineralization. Yet, due to the comparatively small amount of oil addition and the regular ventilation, anoxic conditions are likely to occur only on small microsites but not in the entire incubation vessel. As organic intermediates and CH_4 are usually rapidly re-oxidized to CO_2 when transported through soil (Mancinelli 1995), even an anaerobic degradation mechanisms might finally result in CO_2 release. In addition, rates of anaerobic oil degradation and associated CH_4 evolution are low compared with mineralization to CO_2 (Ward et al. 1980).

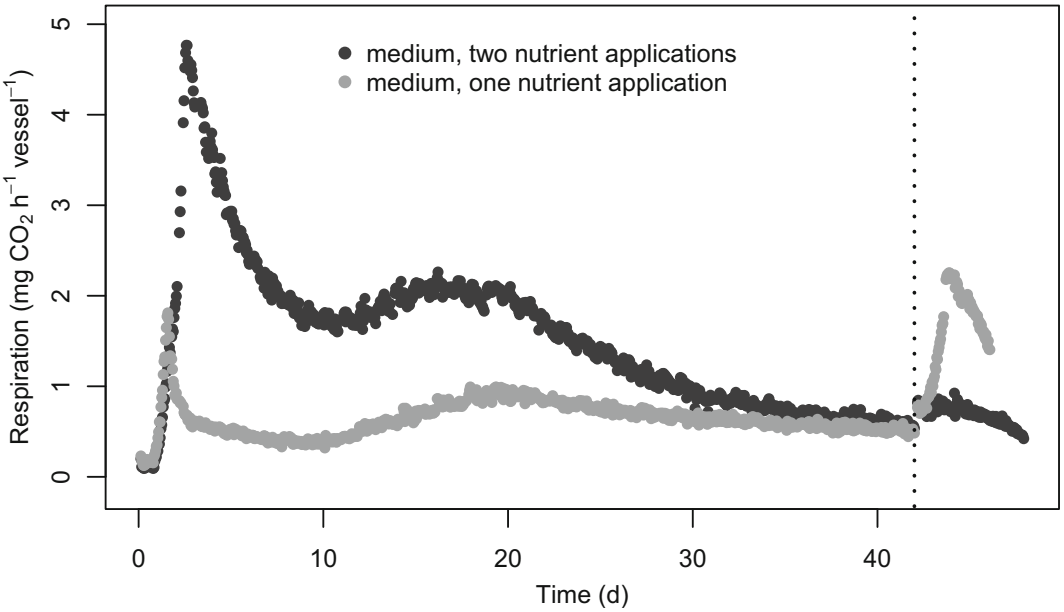

Fig. 3 Effect of nutrient additions on hourly CO_2 release. Samples received either a nutrient mixture (black symbols) or no nutrients (grey symbols) together with the oil at the beginning of incubation (day 0). At day 42, both samples received nutrients either for the second time (black symbols) or for the first time (grey symbols). The curve with black symbols was not exactly congruent after day 42 due to mixing effects. Results are shown exemplarily for the 'medium-dosage' treatment of soil 2

Effect of oil addition on SOC mineralization

The application of vegetable oil resulted in a stimulation of native SOC mineralization, i.e. we observed a priming effect > 1 in all treatments. Kuzyakov et al. (2000) reported priming effects > 1 after adding a wide range of labile C substrates. The addition of labile C may increase the activity of microorganisms, thereby also stimulating the turnover of less easily mineralizable native SOC (Alvarez and Alvarez 2000; Chen et al. 2014; Kuzyakov et al. 2000). As we observed that vegetable oil contains considerable proportions of easily mineralizable C (see above), it seems reasonable to assume that it also stimulates the turnover of SOC.

The driving force behind the priming effect continues to be the subject of controversial discussion. According to Craine et al. (2007), the presence of labile C sources increases the demand for nutrients but provides energy for the breakdown of SOM, which contains the required nutrients. As the breakdown of SOM involves mineralization of C contained within the SOM, the priming effect has correspondingly been interpreted as a response of microbes to nutrient deficiency (Murphy et al. 2015). The development of a second microbial growth phase in the presumably nutrient limited treatments medium dosage and high dosage may thus indicate such a microbial nutrient mining, i.e. a delayed release of previously unavailable nutrients (Meyer et al. 2017; Teklay et al. 2006).

Noteworthy and contrary to the assumption of a deficiency-regulated priming effect, however, the addition of nutrients did not decrease the priming effect. Instead, it even increased priming in the medium-dosage treatment. In line with Meyer et al. (2018), this observation indicates that the demand for nutrients is not the primary mechanism regulating the priming effect, i.e. a release of previously unavailable nutrients from SOM must not be coupled with an increased priming effect. More likely, the oil-induced stimulation of microbial activity seems to enhance the mineralization of native SOC, which is more pronounced at sufficient nutrient supply.

Conclusions

Our study reveals that high amounts of vegetable oil-C are only rapidly mineralized in soil in case of sufficient nutrient supply. At higher application dosages or in nutrient poor soils, nutrient demand may exceed nutrient supply and induce a temporary accumulation of oil in soils. Likewise, it may also be expected that the oil-induced immobilization of nutrients has negative consequences for plant growth. For the stimulation of oil-C mineralization, we therefore recommend fertilizing the soil. The risk of oil accumulation in soil and of priming-induced depletion of SOC stocks needs further investigation by long-term field experiments.

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